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Primary sex ratio adjustment to experimentally reduced male UV attractiveness in blue tits

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The study of primary sex ratio adjustment in birds is notorious for inconsistency of results among studies. To develop our understanding of avian sex ratio variation, experiments that test a priori predictions and the replication of previous studies are essential. We tested if female blue tits *Parus caeruleus* adjust the sex ratio of their offspring to the sexual attractiveness of their mates, as was suggested by a previous benchmark study on the same species. In 2 years, we reduced the ultraviolet (UV) reflectance of the crown feathers of males in the period before egg laying to decrease their attractiveness. In contrast to the simple prediction from sex allocation theory, we found that the overall proportion of male offspring did not differ between broods of UV-reduced and control-treated males. However, in 1 year, the UV treatment influenced offspring sex ratio depending on the natural crown UV reflectance of males before the treatment. The last result confirms the pattern found in the previous blue tit study, which suggests that these complex patterns of primary sex ratio variation are repeatable in this bird species, warranting further research into the adaptive value of blue tit sex ratio adjustment to male UV coloration. *Key words:* blue tit *Parus caeruleus*, male attractiveness, primary sex ratio, sex allocation, ultraviolet plumage. *[Behav Ecol 17:539–546 (2006)]*

Sex allocation theory has been very successful in explaining and predicting patterns of adaptive primary sex ratio variation especially in invertebrate taxa, for example, haplodiploid insects (Godfray and Werren 1997; West et al. 2000). However, when applied to primary sex ratio variation in vertebrates with chromosomal sex determination, such as birds, the explanatory success of sex allocation theory seems modest (Williams 1979; Clutton-Brock 1986; Krackow 2002; Ewen et al. 2004; but see West and Sheldon 2002; West et al. 2005). In birds, females are the heterogametic sex, which potentially gives them control over the sex of the gametes they produce (Krackow 1995; Oddie 1998). However, although the results of many studies follow the predictions of sex allocation theory and therefore suggest adaptive primary sex ratio adjustment in several species of birds (e.g., Dijkstra et al. 1990; Ellegren et al. 1996; Komdeur et al. 1997; Nager et al. 1999; Kalmbach et al. 2001), there are also many studies not providing any evidence for adaptive primary sex ratio variation, despite adequate sample sizes (e.g., Newton and Marquiss 1979; Koenig and Dickinson 1996; Westerdahl et al. 1997; Leech et al. 2001; Budden and Beissinger 2004). Furthermore, results have been inconsistent between studies within the same species and may differ between different years or different populations (e.g., Lessells et al. 1996; Kölliker et al. 1999; Radford and Blakey 2000; Verboven et al. 2002). The notorious inconsistency among avian sex allocation studies has led to critical views on how general a phenomenon adaptive primary sex ratio adjustment is in birds (Radford and Blakey 2000; Komdeur and Pen 2002; Krackow 2002), and several authors have suggested the existence of a publication bias in favor of positive evidence (Hasselquist and Kempenaers 2002; Krackow 2002; Ewen et al. 2004).

Interestingly, two recent meta-analyses of the literature on avian sex ratio variation came to opposing conclusions on the generality of facultative primary sex ratio adjustment in birds. West and Sheldon (2002), who restricted their analysis to studies with clear a priori predictions, concluded that birds can show strong sex ratio shifts. However, Ewen et al. (2004) who conducted a more extensive meta-analysis, which also included studies with weaker a priori predictions, found no evidence for the general occurrence of avian primary sex ratio adjustment. Nevertheless, Ewen et al. (2004) identified a few influential case studies that showed particularly large effect sizes, but it is unclear if these individual studies represent rare biological exceptions in which the study species indeed exhibits sex ratio control or whether these studies represent false positives (i.e., statistical type-I errors). The majority of published avian sex ratio studies to date is correlative (e.g., only 7 out of 40 studies used in the meta-analysis by Ewen et al. (2004) were experimental; see also Komdeur and Pen 2002), and the inclusion of correlative studies giving post hoc adaptive explanations is likely to lead to type-I statistical errors, publication bias, and complications in the application of meta-analysis (Palmer 2000; Gurevitch et al. 2001; West and Sheldon 2002; Ewen et al. 2004). To gain further insight into the avian sex ratio variation, experimental studies that test clear a priori predictions concerning causal relationships between sex ratio and the variables under investigation are needed. Furthermore, it is crucial to replicate key studies to evaluate the robustness and generality of the patterns found (Palmer 2000; Griffith et al. 2003). However, real replicates (i.e., replicates in the same species) of existing studies are scarce (Palmer 2000).

In this paper, we report a replicate of the highly influential benchmark study by Sheldon et al. (1999), which suggested facultative primary sex ratio adjustment in response to experimental variation in male attractiveness in a wild blue tit *Parus caeruleus* population. The study of Sheldon et al. (1999) was based on the hypothesis by Trivers and Willard (1973) that it would be adaptive for individuals to adjust the relative investment in offspring of the different sexes in response to any physiological or ecological variable influencing the relative fitness of sons and daughters. Blue tits mostly form socially monogamous pairs during breeding, but nevertheless, regularly engage in extrapair copulations leading to roughly 10%

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of all offspring being sired by an extrapair male (Kempenaers et al. 1997; Leech et al. 2001; Delhey et al. 2003; P Korsten, CM Lessells, AC Mateman, and J Komdeur, unpublished data). Therefore, female blue tits paired to sexually attractive males are predicted to bias the sex ratio of their offspring towards sons because—given the option of pursuing extrapair matings—sons would benefit more than daughters from inheriting their father's attractiveness. Conversely, females paired to less attractive males should produce female-biased sex ratios as their sons may suffer from increased rates of cuckoldry (Sheldon et al. 1999; see also Burley [1981] for rationale).

The ultraviolet (UV) reflectance of the bright blue crown feathers of male blue tits is an important cue in both social and extrapair mate choice (Andersson et al. 1998; Hunt et al. 1998; Delhey et al. 2003). Furthermore, male survival is positively correlated to male crown UV reflectance, while the proportion of male offspring is positively correlated to male survival (Svensson and Nilsson 1996; Sheldon et al. 1999; Griffith et al. 2003). In line with these findings, Sheldon et al. (1999) found that the proportion of male offspring was positively correlated to natural variation in male crown UV reflectance. However, an experimental reduction of the UV reflectance of males—making them unattractive—before their mates had started egg laying did not lead to a lower proportion of sons in broods of UV-reduced males compared to control males (Sheldon et al. 1999). Instead, the UV reduction reversed the positive correlation between the sex ratio and natural male UV reflectance so that the proportion of sons decreased with increasing pretreatment UV reflectance. This experimental result was unexpected and lacks a good biological explanation as the most straightforward prediction from the hypothesis of Trivers and Willard (1973) is that females paired to UV-reduced—unattractive—males should produce a lower overall proportion of sons than control females (Burley 1981). The results of Sheldon et al. (1999), which were based on a single breeding season, were partly corroborated by correlative data collected in the same population during two additional breeding seasons, but the UV-reduction experiment was not repeated (Griffith et al. 2003). In contrast to the studies above, Leech et al. (2001), who conducted a large-scale study in a different blue tit population and measured male survival, extrapair mating success, and offspring sex ratios—but not crown UV reflectance—found no significant relationships between offspring sex ratio and any of the variables measured. It is unclear whether these inconsistent results reflect genuine differences between blue tit populations or study years or are caused by statistical type-I or type-II errors (Griffith et al. 2003).

The aim of this study was to exactly replicate the study by Sheldon et al. (1999) to evaluate the robustness and generality of the intriguing combined effect of the UV manipulation and male premanipulation UV reflectance on offspring sex ratio. Therefore, we reduced the crown UV reflectance of male blue tits before their mates had started egg laying and determined the resulting offspring sex ratios in 2 years, while closely following the experimental protocol of Sheldon et al. (1999). We tested 1) if overall sex ratio was more female biased for the UV reduced than for the control group as predicted by the Trivers and Willard (1973) hypothesis (Burley 1981) and 2) if the effect of the male UV treatment on sex ratio depended on UV reflectance before treatment as was found by Sheldon et al. (1999).

METHODS

Study population and general field methods

We carried out the UV manipulation experiment in a nest-box population of blue tits in "De Vosbergen" (53°08′N, 06°35′E) near Groningen, the Netherlands, during the breeding seasons of 2002 and 2003. The blue tit breeding population in De Vosbergen was monitored from 2001 to 2003. During this period, all breeding adults were captured in their nest-boxes when feeding nestlings, mostly between days 6 and 14 (where day of hatching of the first nestling = day 0). We sexed adults by the presence (=female) or absence (=male) of an incubation patch and aged them as first year or older (see Svensson 1992). We also measured mass (±0.1 g), length of tarsus (±0.1 mm) and third primary feather (±0.5 mm), and the reflectance of the crown feathers (see below for details on the procedure). We ringed and blood sampled all adults caught. We took small blood samples (ca., 10 μl) from the nestlings on day 4, and unhatched eggs and nestlings found dead before blood sampling were also collected for molecular determination of sex using the P2 and P8 primers of Griffiths et al. (1998). Molecular sexes of 81 nestlings and 231 adults were confirmed (no mismatches) using field observations of the same individuals when breeding.

Measurements of crown reflectance

We measured the reflectance of the crown feathers using a USB-2000 spectrophotometer with a DH-2000 deuterium–halogen light source (both Avantes, Eerbeek, The Netherlands). The measuring probe was held at a right angle against the plumage, that is, both illumination and recording were at 90° to the feathers. During each crown reflectance measurement, we took five replicate readings and smoothed each of these reflectance spectra by calculating the running mean over 10-nm intervals. Following previous studies of UV color signaling in blue tits (e.g., Sheldon et al. 1999; Delhey et al. 2003; Griffith et al. 2003), we calculated indices of the three main dimensions of color perception—brightness, hue, and chroma (Hailman 1977)—and averaged these across the five replicate spectra. Brightness (spectral intensity) was the sum of reflectance between 320 and 700 nm ($R_{320–700}$), which corresponds to the spectral range visible to blue tits (Hart et al. 2000). Hue (spectral location) was the wavelength of maximum reflectance, $\lambda(R_{max})$. As an index of chroma (spectral purity), we used "UV chroma," which was the sum of reflectance between 320 and 400 nm divided by the sum of reflectance between 320 and 700 nm ($R_{320–400}/R_{320–700}$). Crown color measurements were repeatable within individuals between separate days of capture within a single breeding season (mean number of days between captures: 4.1 ± 2.4 SD; repeatability brightness = 0.60, $F_{14,15} = 4.04$, $P = 0.006$; repeatability hue = 0.65, $F_{14,15} = 4.74$, $P = 0.002$; repeatability UV chroma = 0.70, $F_{14,15} = 6.02$, $P < 0.001$; see Lessells and Boag 1987).

Manipulation of male crown UV reflectance

We manipulated the crown UV reflectance of male blue tits before their females started egg laying. Males were captured near nest-boxes containing completed nests with mist nets using song playback and a mounted male blue tit specimen as a decoy. Males were assigned sequentially to the UV reduction or control treatment. To reduce the crown UV reflectance, we used a previously developed method (Andersson and Amundsen 1997; Sheldon et al. 1999; Limbourg et al. 2004) in which a mixture of UV-blocking chemicals (Parsol 1789 and MCX, Roche, Switzerland) and duck preen gland fat (fishing fly dressing, purchased at Euro-Fly, Paris, France) was applied to the males' crown feathers. As a control treatment, we applied duck preen gland fat only. Crown reflectance was measured immediately before and after the treatment. The UV-reduction treatment was effective in reducing UV

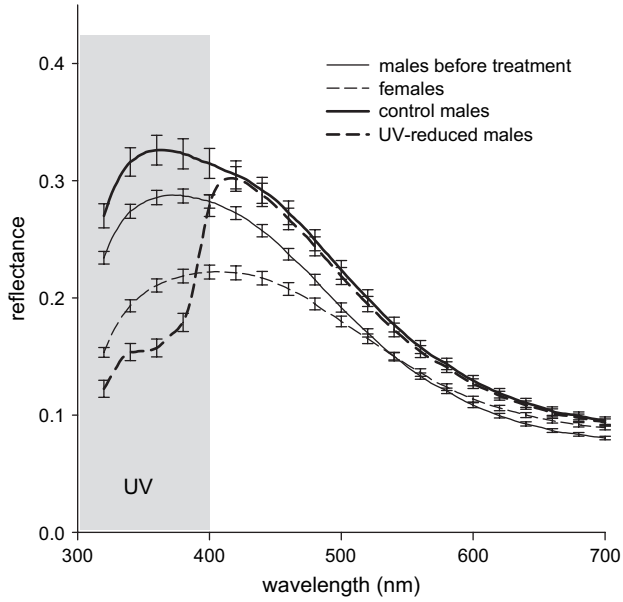

Figure 1
Mean crown reflectance curves of male blue tits before UV manipulation ($n = 70$), after UV reduction ($n = 37$), and after control treatment ($n = 31$) and of female blue tits captured during nestling provisioning ($n = 70$), for the years 2002 and 2003 combined. Crown reflectance of two control-treated males was not remeasured after treatment. Standard errors around the means are depicted at 20-nm intervals. The shaded area indicates the UV part of the spectrum.

reflectance (comparison with pretreatment UV chroma: paired $t_{36} = 30.0$, $P < 0.0001$), whereas the control treatment did not affect UV reflectance (UV chroma: paired $t_{30} = 1.56$, $P = 0.13$; Figure 1). The gloss of the preen fat produced a slight uniform increase in reflectance in both treatments (Figure 1). The UV reduction caused by the UV-reduction treatment is known to decrease with time but was still detectable after about 10 days in a previous study on blue tits using the same technique (Limbourg et al. 2004). During nestling feeding (ca., 30–50 days after the treatment), there was no longer a difference between UV-reduced and control-treated males (P Korsten and J Komdeur, unpublished data). Because the effect of the UV-reduction treatment diminished over time, we aimed to have a similar interval between male UV treatment and laying of the first egg by their mates as Sheldon et al. (1999) (Sheldon et al. 1999: 10 days ± 5.2 SD; this study: 2002: 4.4 days ± 2.9 SD; 2003: 8.0 days ± 6.5 SD).

We manipulated the crown UV reflectance of 84 males, 35 in 2002 and 49 in 2003. Of these, 70 males were included in our analyses: 26 in 2002 (13 UV reduced, 13 control) and 44 in 2003 (24 UV reduced, 20 control). The other UV-manipulated males were excluded for the following reasons: seven males were not recaptured during nestling provisioning and could therefore not be assigned with certainty to a specific brood, five males turned out to have been UV manipulated after their female had started egg laying, one male was polygynous, and the clutch of one male was destroyed before hatching due to vandalism. Males of both treatment groups did not differ in age (Yates' corrected $\chi^2_1 = 0.826$, $P = 0.36$), body size (mass and tarsus and third primary length: t-tests, all $P > 0.33$), or pretreatment crown color (brightness, hue, UV chroma: t-tests, all $P > 0.72$). There was also no difference between the treatment groups in subsequent clutch size ($t_{68} = 0.12$, $P = 0.90$) or laying date of the first egg ($t_{68} = 0.16$, $P = 0.87$). On 18 occasions, we also captured a female when mistnetting the male. Both members of all these putative pairs were recaptured in the same nest-box when provisioning the nestlings, indicating that pair formation had taken place before the experimental treatment and that the treatment did not lead to divorce.

Data analyses

To test whether the distributions of sex ratios (i.e., the proportions of sons) over broods departed from binomial, we conducted randomization tests. These were carried out by randomly redistributing the nestlings over the broods 10 000 times, while keeping the original distribution of brood sizes, and calculating the deviance each time. The P value was obtained from the proportion of the 10 000 runs in which the deviance was greater than for the real broods. We used multilevel mixed models with a binomial error distribution with a logit link function following Krackow and Tkadlec (2001) and Rasbash et al. (2004) to analyze sex ratio, with nestlings nested within broods (i.e., brood identity was fitted as a random effect). The models were implemented using restricted iterative generalized least squares and second-order penalized quasi-likelihood approximation (Rasbash et al. 2004). To test for a main effect of the UV treatment on sex ratio (Trivers and Willard 1973; Burley 1981), we fitted UV treatment, year (2002, 2003), and their interaction (see Figure 2). To test if the effect of UV treatment was dependent on pretreatment male crown color (Sheldon et al. 1999), we fitted

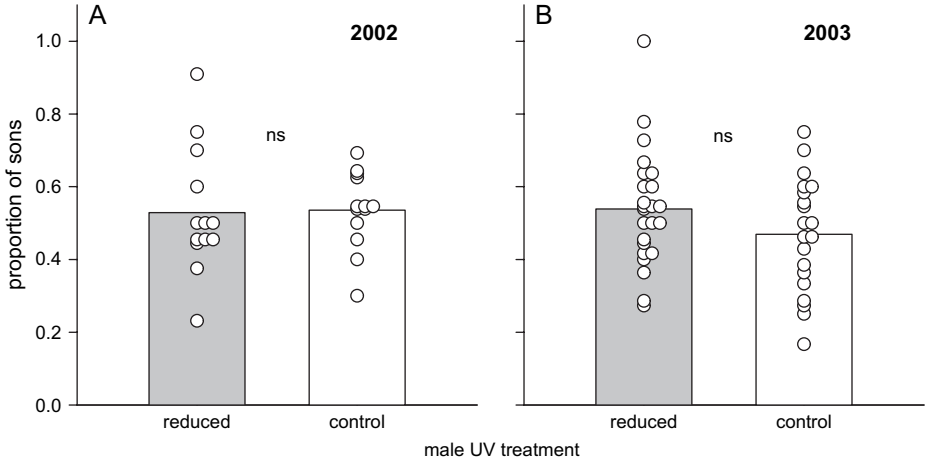

Figure 2
No difference in the proportion of male offspring in broods of UV-reduced and control-treated male blue tits in 2002 (A) or 2003 (B) ($n = 70$; UV treatment: Wald = 1.383, df = 1, $P = 0.24$; year: Wald = 1.418, df = 1, $P = 0.23$; UV treatment × year: Wald = 0.927, df = 1, $P = 0.34$). Bars indicate mean proportion of male offspring for each experimental group. Circles indicate proportions of male offspring of individual broods.

Table 1
Multilevel models of brood sex ratios of blue tits

Variables included in models	Brightness		Hue		UV chroma	
	Wald	*P*	Wald	*P*	Wald	*P*
UV treatment	0.016	0.90	0.947	0.33	7.717	0.0055
Color index	0.011	0.92	1.556	0.21	5.875	0.0154
Year	0.148	0.70	2.524	0.11	8.279	0.0040
UV treatment × year	0.814	0.37	0.767	0.38	7.365	0.0067
Color index × year	0.046	0.83	2.647	0.10	7.939	0.0048
Color index × UV treatment	0.090	0.76	0.995	0.32	7.444	0.0064
Color index × UV treatment × year	1.144	0.28	0.836	0.36	7.006	0.0081

UV treatment, year (2002 or 2003), male color index (brightness, hue, UV chroma), and all their interaction terms were retained in all models (all $n = 70$). The table shows Wald statistics and *P* values. All df = 1. See also Figure 4.

UV treatment, year, pretreatment crown color (brightness, hue, UV chroma), and all interactions (see Table 1; Figure 4). As two of the three crown color indices were strongly intercorrelated (hue vs. UV chroma: $r_p = -0.76$, $n = 70$, $P < 0.001$), we fitted independent models for each of the crown color indices. The significance of variables was tested using the Wald statistic, which follows a chi-square distribution. Five males and nine females belonged to experimental pairs ($n = 10$ pairs) both in 2002 and 2003. Proportions of male offspring were not significantly correlated between years within these individuals either in males ($r_s = 0.67$, $n = 5$, $P = 0.22$) or females ($r_s = 0.29$, $n = 9$, $P = 0.44$), and we included these individuals in our analyses for both years. Multilevel models were carried out using MLwiN 2.0 and all other statistical tests using SPSS 12.01. *P* values are two-tailed.

RESULTS

Sex ratio variation at the population level

We sexed 95.3% of eggs laid ($n = 783$) in 70 experimental broods and 96.6% of eggs laid ($n = 292$) in 26 nonexperimental broods used in within-individual comparisons (see below). In total, we collected 44 of 71 unhatched eggs of which we sexed 29 embryos. There was no visible embryo development in the majority of the eggs that were not sexed. We found no indication for sex-biased embryo mortality; 41.4% ($n = 29$) of sexed embryos were male versus 51.0% ($n = 999$) of all sexed nestlings (Yates' corrected $\chi^2_1 = 0.69$, $P = 0.41$). Therefore, we assumed that our data represent brood sex ratios at laying (i.e., the primary sex ratio).

Overall, 51.6% ($n = 746$) of offspring in experimental and 48.2% ($n = 282$) of offspring in nonexperimental broods were male, which in neither case differed from 50% (experimental broods: Yates' corrected $\chi^2_1 = 0.71$, $P = 0.40$; nonexperimental: Yates' corrected $\chi^2_1 = 0.29$, $P = 0.59$). The distribution of male and female offspring over broods also did not depart from a binomial distribution in either experimental (randomization test: deviance = 74.97, degrees of freedom [df] = 69, $P = 0.42$) or nonexperimental broods (randomization test: deviance = 22.82, df = 25, $P = 0.65$).

Main effect of UV treatment on sex ratio

There was no difference in the overall proportion of male offspring between the UV-reduced and the control-treated group in either 2002 or 2003 (Figure 2). Neither was there any suggestion of a main effect of experimental treatment on sex ratio from the limited sample of individuals for which within-individual comparisons were possible (Figure 3; see Oddie and Reim [2002] for an explanation why such tests may be more powerful).

Effect of pretreatment male UV reflectance on sex ratio

Sex ratios were not related to either pretreatment crown brightness or hue of males (Table 1; Figure 4A,B,D,E). However, the interaction term "UV treatment × pretreatment UV chroma × year" had a significant effect on sex ratio (Table 1). This three-way interaction including "year" indicates that the interacting effects of UV treatment and pretreatment UV chroma differed between the 2 years. To analyze this effect in more detail, we fitted the effects of UV treatment and pretreatment UV chroma and their interaction separately for each year. In 2003, but not in 2002, we found that the interaction of UV treatment with pretreatment UV chroma had a significant effect on sex ratio (UV treatment × UV chroma—2002: Wald = 1.16, df = 1, $n = 26$, $P = 0.28$; 2003: Wald = 7.44, df = 1, $n = 44$, $P = 0.0064$; Figure 4C,F). Subsequent analysis revealed that in 2003, pretreatment UV chroma was positively related to the proportion of sons in the control group (Wald = 5.880, df = 1, $n = 20$, $P = 0.015$) but not related to the proportion of sons in the UV-reduced group (Wald = 2.177, df = 1, $n = 24$, $P = 0.14$) (Figure 4F).

DISCUSSION

No main effect of UV treatment

In contrast to previous suggestions from correlative data (Sheldon et al. 1999; Griffith et al. 2003), we found no experimental evidence for primary sex ratio adjustment in relation to male (UV) attractiveness in blue tits according to the simple prediction from sex allocation theory (Trivers and Willard 1973; Burley 1981). Although the UV-reduction treatment we used has been shown to effectively lower attractiveness of male blue tits (Limbourg et al. 2004), we found no difference between the overall sex ratios produced by females paired to UV-reduced and control-treated males. This result confirms the result of Sheldon et al. (1999) who also found no difference in overall sex ratio between the UV-reduced and control-treated groups. Likewise, Foerster and Kempenaers (2004) who experimentally enhanced the attractiveness of male blue tits by testosterone-releasing implants found no difference between overall sex ratio produced by females paired to testosterone-implanted—attractive—and control males. Only

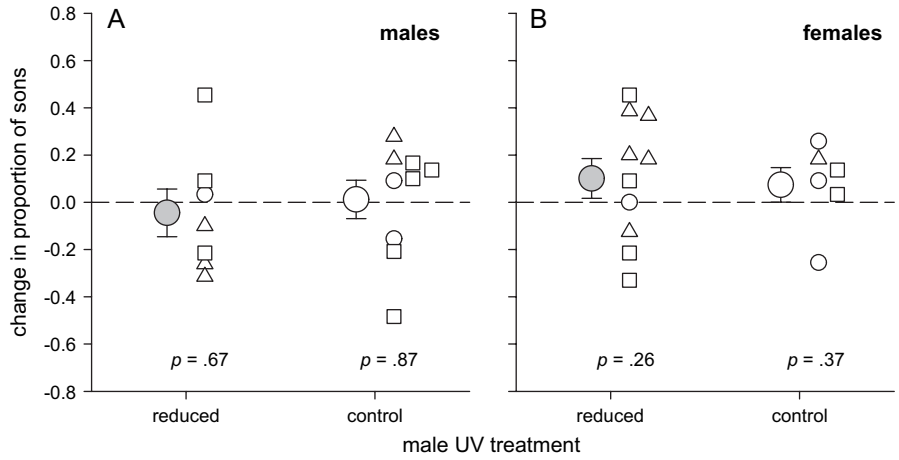

Figure 3
Within-individual comparisons of brood sex ratio for males (A) and females (B) recorded breeding in 2 years; one in which they were subject to the experiment and one in which they were not (change in proportion of sons = proportion of sons in experimental brood − proportion of sons in nonexperimental brood). Large circles are mean changes with standard errors. Small symbols indicate within-individual changes—circles: nonexperimental brood in 2001, experimental brood in 2002; triangles: experimental brood in 2002, nonexperimental brood in 2003; squares: nonexperimental brood in 2002, experimental brood in 2003. The within-individual changes in sex ratio did not differ from 0 (one-sample *t*-tests—UV-reduced males: $t_6 = -0.445$, $P = 0.67$; control males: $t_8 = 0.165$, $P = 0.87$; females paired to UV-reduced males: $t_9 = 1.198$, $P = 0.26$; females paired to control males: $t_5 = 0.992$, $P = 0.37$). Furthermore, the within-individual change in sex ratio did not differ between UV-reduced and control broods (ANOVA—males: $F_{1,12} = 0.126$, $P = 0.73$; females: $F_{1,12} = 0.085$, $P = 0.78$), and the magnitude of the change was not dependent on the year of experimental treatment (ANOVA—males: $F_{1,12} = 0.009$, $P = 0.93$; females: $F_{1,12} = 0.018$, $P = 0.90$), or on the sequence of the experimental and nonexperimental year (ANOVA—males: $F_{1,12} = 0.010$, $P = 0.92$; females: $F_{1,12} = 1.299$, $P = 0.28$).

two experimental studies in other bird species in which some aspect of male attractiveness was manipulated have found an effect on primary sex ratio such as predicted by the Trivers and Willard (1973) hypothesis (collared flycatcher [*Ficedula albicollis*], Ellegren et al. 1996; spotless starling [*Sturnus unicolor*], Polo et al. 2004). Several other experimental studies in different species failed to find an effect of male attractiveness on primary sex ratio (barn swallow [*Hirunda rustica*], Saino

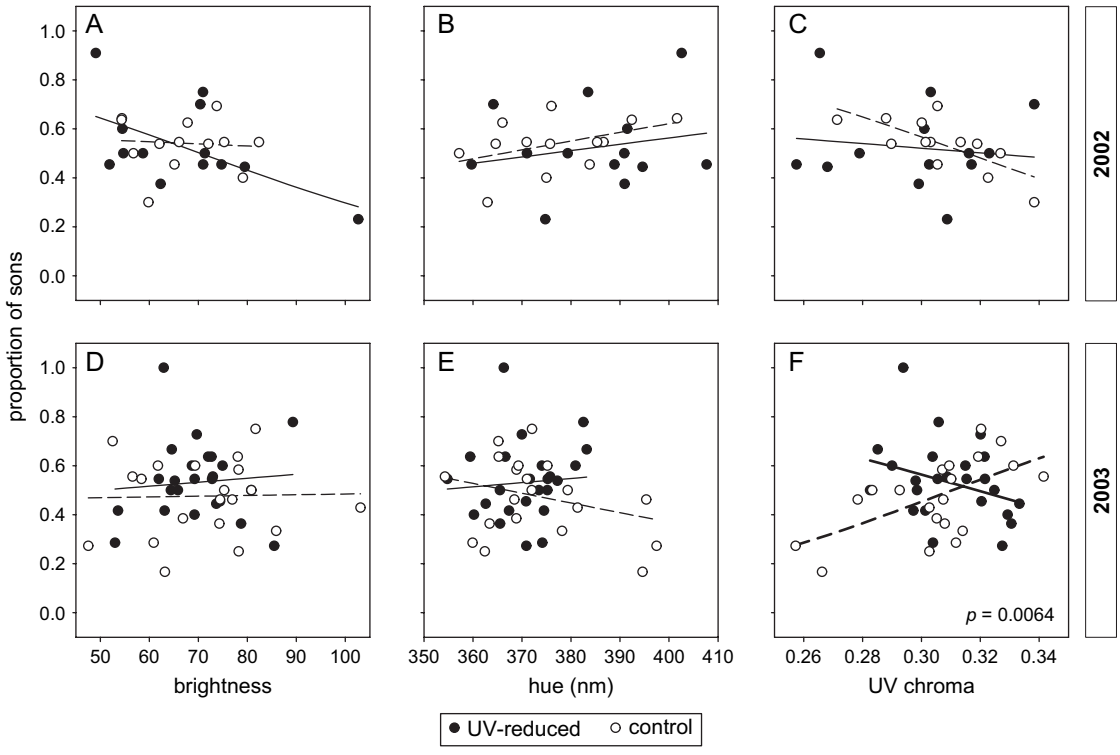

Figure 4
Relationships between brood sex ratio and male pretreatment crown brightness (A, D), hue (B, E), and UV chroma (A, B) for blue tits of two treatment groups (UV reduced and control) in 2002 (A–C) and 2003 (D–F). Solid and dashed lines indicate the predicted proportions of sons in UV reduced and control broods, respectively, depending on male pretreatment brightness, hue, and UV chroma. Nonsignificant relationships are indicated by thin lines (A–E), whereas thick lines indicate statistically significant relationships (F). See also Table 1.

et al. 1999; mallard [*Anas platyrhynchos*], Cunningham and Russell 2000; dark-eyed junco [*Junco hyemalis*], Grindstaff et al. 2001; zebra finch [*Taeniopygia guttata*], Rutstein et al. 2004; Zann and Runciman 2003).

Interaction of UV treatment and pretreatment UV

Although there was no main effect of UV treatment on sex ratio, the interaction of premanipulation UV chroma with UV treatment had a significant effect on sex ratio in 2003 but not in 2002. This significant interaction effect was caused by the presence of a significantly positive relationship between the proportion of sons and male premanipulation UV chroma in the control group, whereas such a relationship was absent in the UV-reduced group. Our result is almost identical to the pattern found by Sheldon et al. (1999), although Sheldon et al. (1999) also found the interaction of premanipulation hue—which is negatively correlated with the UV chroma index—with UV treatment to be significant. As a causal explanation for the curious interaction effect of UV treatment and pretreatment crown color, which does not follow the initial prediction of an overall bias toward daughters in the UV-reduced group (Burley 1981), Sheldon et al. (1999) proposed that the UV-reduction treatment might not merely make males unattractive but may completely mask the variation in natural male UV reflectance. Possibly, this deprives females of cues on male UV attractiveness, leading to the absence of a relation between male UV and sex ratio which is naturally present in the control group of males with unaffected UV reflectance. In addition, several other factors could be important for the female's perception of her mate's attractiveness/quality after the UV-reduction treatment, such as the discordance between the reduced UV reflectance of the crown feathers and the UV reflectance of other plumage parts, for example, the UV/blue wing coverts, which are correlated in unmanipulated birds (Sheldon et al. 1999; our study, crown vs. wing coverts UV chroma: $r_p = 0.45$, $n = 48$, $P = 0.001$), or other male quality signals such as song performance. We also suggest that the UV reduction may interfere with the signaling function of the male crown plumage during male–male territorial conflicts (Alonso-Alvarez et al. 2004), which could also influence the female's perception of the quality of her mate. All of these possible explanations are speculative, and carefully designed experiments and detailed behavioral observations of UV-manipulated individuals are needed to better understand the biological consequences of the UV treatment.

It is unclear why we found the interaction of pretreatment UV chroma and UV treatment to have a significant effect on sex ratio in 2003 but not in 2002. Possibly, the interval between UV manipulation and laying of the first egg was too short in 2002 (4.4 days ± 2.9 SD), whereas in 2003, this interval was longer (8.0 ± 6.5 SD) and closer to the interval in the study of Sheldon et al. (1999) (10 days ± 5.2 SD). In both years, we aimed to have a similar interval between treatment and egg laying as Sheldon et al. (1999), but due to the unpredictability of the onset of egg laying, it is impossible to achieve a fixed interval between treatment and subsequent egg laying. Females may need a minimum amount of time to influence offspring sex ratios, or there may be a time window during which females are particularly sensitive to the appearance of their mates. Given that the UV-reducing effect diminishes over time, the timing of the experimental treatment with respect to subsequent egg laying is probably crucial for the treatment to influence the sex ratio. However, this idea was not supported by a significant effect of the interaction of the interval between UV treatment and laying of the first egg × UV treatment × premanipulation UV chroma in either 2002 (Wald = 0.328, $n = 26$, df = 1, all P values = 0.57) or 2003 (Wald = 1.152, $n = 44$, df = 1, $P = 0.28$), but statistical power was low in these analyses. At present, we have no alternative plausible explanations for the significant interaction with year. The two breeding seasons appeared very similar and were not different in, for example, mean laying date ($t_{68} = -1.286$, $P = 0.20$), clutch size ($t_{68} = 0.025$, $P = 0.98$), or fledging success (Wald = 0.170, $n = 70$, df = 1, $P = 0.68$). Also female body condition, which can also influence primary sex ratio (Nager et al. 1999), was not different between the 2 years (measured as body mass, controlled for tarsus length; ANOVA: $F_{1,67} = 0.162$, $P = 0.69$).

Interannual variation in patterns of primary sex ratio among individual broods has more often been encountered in birds, but most studies lack convincing biological explanations for such year effects (Lessells et al. 1996, Hartley et al. 1999; Korpimäki et al. 2000; Radford and Blakey 2000; Griffith et al. 2003; CM Lessells, unpublished data). Population-wide sex ratios, however, have previously been shown to vary among years in relation to food availability (e.g., Wiebe and Bortolotti 1992; Hipkiss and Hörnfeldt 2004), length of the breeding season (Weatherhead 2005), and the mean number of helpers at the nest (Dickinson 2004).

Adaptive sex ratio adjustment

Several studies reporting strong biases in primary sex ratio show that birds can have considerable control over offspring sex ratio (e.g., Heinsohn et al. 1997; Komdeur et al. 1997, 2002). Furthermore, patterns of avian primary sex ratio variation may be very flexible to variable selective pressures (Badyaev et al. 2002; Zann and Runciman 2003) and can be complex (Legge et al. 2001). Therefore, we believe that the complex relationship between blue tit sex ratio and male attractiveness that we found, which did not follow simple prediction from theory (Trivers and Willard 1973; Burley 1981) and seems rather inconsistent between study populations and years (Sheldon et al. 1999; Leech et al. 2001; Griffith et al. 2003; our study), does not necessarily indicate that blue tit primary sex ratio variation is nonadaptive or constrained by their chromosomal sex determination system. It rather suggests that simple verbal arguments (Burley 1981) predicting optimal sex ratio in relation to mate attractiveness may not generally be applicable (Pen and Weissing 2000). Patterns of optimal sex ratio variation may be subtle and vary between years and populations, depending on the local ecological circumstances.

The relationship between the optimal offspring sex ratio, yielding maximum fitness, and paternal attractiveness may be relatively weak and/or not straightforward in blue tits for several reasons. Firstly, the typical percentage of approximately 10% extrapair offspring in blue tits (e.g., Kempenaers et al. 1997; Delhey et al. 2003; P Korsten, CM Lessells, AC Mateman, and J Komdeur, unpublished data for the present study population) is not particularly high when compared to other socially monogamous bird species (Griffith et al. 2002). The variance in reproductive success sets an upper limit to the strength of sexual selection and, hence, of selection on sex ratio in relation to male sexual attractiveness (cf., Griffin et al. [2005] for variation in the extent of sex ratio modification in relation to the strength of selection through benefits from helpers at the nest). The strength of selection on sex ratio variation may thus be relatively low in blue tits. This may especially be true when compared to, for example, ungulate mammals with harem systems that often do show the patterns of sex ratio variation predicted from the Trivers and Willard (1973) hypothesis (Sheldon and West 2004). In many of these ungulate species, there is extreme variation in reproductive success among males, with individual males being either very

successful or unsuccessful, while most females have relatively similar reproductive output.

Another reason why the relationship between the optimal sex ratio and male crown color may not be simple in blue tits is that the relationship between male within and extrapair mating success and UV crown coloration is not necessarily straightforward. It has recently been found that males with more UV-shifted crown reflectance are less cuckolded, whereas males with less UV-shifted crown reflectance sire more extrapair young (Delhey et al. 2003). However, this result is based on a single breeding season and seems to contrast with a previous study, in a different blue tit population, which suggests that males that are successful in gaining extrapair paternity also have a higher share of paternity in their own broods (Kempenaers et al. 1997). Thus, it remains to be investigated how the relative reproductive values of sons and daughters depend on the crown UV reflectance of their fathers.

Finally, blue tits lay very large clutches (mean clutch size [2001–2003]: 10.9 $\pm$ 1.7 SD, $n = 249$). Until now, most case studies in birds that found strong shifts in primary sex ratio involved species that lay only one or two eggs per clutch (Ewen et al. 2004), suggesting that selection for extreme sex ratio shifts is more constrained by the chromosomal sex determination system (Emlen 1997; Pike 2005; but see Komdeur et al. 2002) and/or weaker in species with large clutches such as the blue tit.

In conclusion, we found no experimental evidence for primary sex ratio adjustment in relation to male UV attractiveness according to the simple prediction from sex allocation theory (Trivers and Willard 1973; Burley 1981). However, remarkably, one of our year's results confirms the intriguing UV treatment $\times$ pretreatment UV interaction effect on sex ratio previously found by Sheldon et al. (1999). This is an extremely important result because it demonstrates that the previously unexpected pattern that Sheldon et al. (1999) found is more than an anomalous result in a single year and population. Our replication of the result therefore provides the basis for moving on to the next step of elucidating the adaptive value of blue tit sex ratio variation and the proximate and ultimate causes of the variability of blue tit sex ratio patterns among years and populations.

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